



## **Bacterial communities of cryoconite holes of the Forni Glacier (Italian Alps) show both seasonal trends and year-to-year variability**

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Cryoconite holes are small depressions filled with water that form on ice surface due to the melting action of dark wind-blown debris warmed by solar radiation. These environments are considered hot-spots of biodiversity and biological activities in glacier environments and host communities dominated by bacteria. The majority of studies on cryoconite holes are based on snapshot sampling, under the implicit assumption that the communities living in these environments are stable. However, some evidence of seasonal variation in cryoconite hole ecological communities exist. In this paper, we modelled the variation in the bacterial communities of cryoconite holes of Forni Glacier (Italian Alps) during the melting seasons of 2013 and 2016, for which samples at three and five time-points were available. Bacterial communities were characterized by high-throughput ILLUMINA sequencing of the hypervariable V5-V6 region of 16S rRNA genes. We found consistent trends in bacterial communities, which shifted from cyanobacteria-dominated communities at the beginning of the melting season in July to communities dominated by heterotrophic orders, particularly Sphingomonadales and Burkholderiales at the end of the melting season in September. Bacterial communities seem therefore to change according to an ecological succession. Seasonal and year-to-year variation in ecological variables, namely cumulated above-zero °C temperatures (positive degree-days, hereafter DD) and cumulated short-wave incoming radiation (hereafter c-SWIN) measured by an automated weather station (AWS-1) located on the Forni Glacier close (< 500 m) to the study area, explained a small but significant amount of variation in bacterial communities. This suggests that variation in insolation can be an important driver of seasonal changes of bacterial communities in these environments. However, difference between years was always highly significant, and explained a large fraction of variance in all the analyses, including those accounting for variation in ecological conditions, suggesting that other factors, not accounted for in this study, concur in determining the structure of bacterial communities found in cryoconite holes. Bacterial communities in cryoconite holes of the Forni Glacier are therefore not stable, but show both seasonal succession and year-to-year variation. Snapshot studies can therefore only partly account for the variation of the bacterial communities of cryoconite holes of temperate glaciers.